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KIRSTI LAHTI

Cyanobacterial hepatotoxins and drinking water supplies – aspects of monitoring and potential health risks

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4

Kirsti Lahti

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Contents

List of original publications

This thesis is based on the following publications, which are referred to by their Roman numerals in the text. In addition, some previously unpublished results are included in the thesis.

I Lahti, K. & L. Hiisvirta 1995. Causes of water-borne outbreaks in community water systems in Finland: 1980–1992. *Water Science and Technology* 31(5–6): 33–36.

II Lepistö, L., K. Lahti, M. Färdig & J. Niemi 1994. Removal of cyanobacteria and other phytoplankton in four Finnish waterworks. *Archiv für Hydrobiologie/Supplement 105 Algological Studies* 75:167–181.

III Lahti, K., J. Ahtiainen, J. Rapala, K. Sivonen & S.I. Niemelä 1995. Assessment of rapid bioassays for detecting cyanobacterial toxicity. *Letters in Applied Microbiology* 21:109–114.

IV Rapala, J., K. Lahti, K. Sivonen & S.I. Niemelä 1994. Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. *Letters in Applied Microbiology* 19:423–428.

V Lahti, K., J. Rapala, M. Färdig, M. Niemelä & K. Sivonen 1997. Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. *Water Research* 31(5):1005–1012.

VI Lahti, K., J. Kilponen, A.-L. Kivimäki, K. Erkomaa & K. Sivonen 1996. Removal of cyanobacteria and their hepatotoxins from raw water in soil and sediment columns. In: *Artificial Recharge of Groundwater* (Eds. Kivimäki, A.-L. & T. Suokko), *Nordic Hydrological Programme Report* 38:187–195.

Cyanobacterial hepatotoxins and drinking water supplies – aspects of monitoring and potential health risks

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Lahti K. 1997. Cyanobacterial hepatotoxins and drinking water supplies – aspects of monitoring and potential health risks. Monographs of the Boreal Environment Research No. 4, 1997.

The aim of this study was to evaluate the risks induced by mass occurrences of hepatotoxic cyanobacteria for the hygienic quality of drinking water in Finland, as well as some aspects of monitoring toxin occurrence in water sources. In this study the efficiencies of four waterworks in the removal of cyanobacteria and possible toxins were investigated. Laboratory experiments were also performed in order to assess the removal of cyanobacterial cells and hepatotoxins during filtration through soil and sediment columns. The waterworks without chemical coagulation treatment were inefficient in the removal of phytoplankton and especially cyanobacteria from raw water, even when filtration through activated carbon was in use. The average removal of cyanobacteria in waterworks with powdered activated carbon, chemical coagulation, sedimentation and sand filtration treatment was 99.9 %. Even with these conventional surface water treatment systems problems may occur during mass occurrence of cyanobacteria unless additional treatment methods are in use. Efficient removal of microcystins from raw water infiltrated through soil and sediment columns was detected except with very high biomass and toxin concentrations. Laboratory experiments with sediments collected from different lakes showed that bacteria capable of microcystin degradation occur in lake sediments, but that the lag periods preceding the onset of degradation of microcystins appear to vary. With sediments from lakes with a recent history of cyanobacterial blooms, degradation started faster than with sediments taken from a humic lake without numerous cyanobacteria. Some bacteria capable of degrading microcystins were isolated from the sediment of lake Tuusulanjärvi. These strains degraded microcystins as rapidly as the microbes present in added sediments, with 90 % removal in less than one week. The studies in lake Tuusulanjärvi indicated much longer persistence of microcystin-LR in water than would have been expected from the laboratory experiments. The decimal reduction time for microcystin-LR in particulate material was about two weeks and for dissolved toxin one month. In comparison of simple toxicity tests to replace mouse bioassay *Artemia salina* bioassay appeared promising for screening of toxicity due to hepatotoxins and anatoxin-a. The luminescent bacteria test was not suitable for this purpose. The results of this study indicate the need to monitor cyanobacterial toxins in raw water and drinking water and to promote methods for the reduction of blooms and toxins.

Keywords: cyanobacteria, microcystins, drinking water, waterworks, filtration, persistence, bioassays, monitoring

1 Introduction

Cyanobacteria are among the earliest known living organisms on earth. They occur in a wide range of habitats from ice fields to hot springs and deserts. Most cyanobacterial species are free-living in freshwaters or in marine or terrestrial environments, whereas a few species are endosymbionts of eukaryotes (Holt *et al.* 1994). Mass occurrences (or blooms) of planktonic cyanobacteria commonly occur especially in eutrophic fresh or brackish water bodies. The major bloom-forming cyanobacterial genera include one or several species with strains that may produce toxins harmful to animals and humans (Gorham and Carmichael 1980, Carmichael 1992a).

Cyanobacteria were earlier classified as blue-green algae according to the Botanical Code (Stafleu *et al.* 1972), but they were assigned to the prokaryotes in the eighth edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974). Cyanobacteria and prochlorophytes are the only groups of prokaryotes having the ability to carry out oxygenic photosynthesis as in all photosynthetic eukaryotic organisms (Stanier and Cohen-Bazire 1977). The classification of cyanobacteria is still in transition due to the limited number of pure cultures available and hence the lack of phylogenetic information of different species (Holt *et al.* 1994). In practice, the identification of cyanobacteria from collected samples is still mainly based on traditional morphological features of cells and colonies as well as on the mode of reproduction (Komárek 1991, Skulberg *et al.* 1993). This morphological identification system is also used in this work.

This study focuses on the problems caused by cyanobacterial hepatotoxins in drinking water supplies. Other toxins produced by cyanobacteria are only briefly reviewed.

1.1 Toxins produced by cyanobacteria

Cyanobacteria produce many secondary metabolites, such as cytotoxins (antibiotics, anticancer compounds) and biotoxins (Carmichael 1992b). In fresh water sources many common cyanobacterial genera, e.g. *Anabaena, Aphanizomenon, Microcystic, Oscillatoria* and *Nostoc,* produce biotoxins which can be divided into neurotoxins and peptide hepatotoxins (Carmichael 1992a). Hepatotoxic water blooms occur worldwide (Carmichael 1992a), whereas neurotoxic cyanobacteria have been reported from North America (Carmichael and Gorham 1978, Repavich *et al.* 1990), Nordic countries (Sivonen *et al.* 1989a, Skulberg *et al.* 1992), United Kingdom (Edwards *et al.* 1992) and Australia (Baker and Humpage 1994). In systematic surveys around the world 25 to 70 % of cyanobacterial water blooms have been toxic (e.g. Carmichael *et al.* 1988b, Pearson *et al.* 1990, Repavich *et al.* 1990, Sivonen 1990a, Skulberg *et al.* 1994). These water blooms have caused acute poisoning of cattle, sheep, horses, pigs, ducks and other wild and domestic animals (e.g. Sivonen 1990a, Carmichael 1992a). In cases of neurotoxic cyanobacteria animals have shown signs of staggering, gasping, convulsions, opisthotonos in birds and increased salivation. Death has often been due to respiratory arrest within minutes or a few hours (Beasley *et al.* 1989, Carmichael 1992a). In the case of hepatotoxic cyanobacteria animals suffer from weakness, anorexia, pallor of mucous membranes, vomiting and diarrhoea. Death occurs within a few hours to a few days, usually as a result of intrahepatic haemorrhage and hypovolemic shock (Theiss and Carmichael 1986, Beasley *et al.* 1989). There are some reports on adverse human health effects, such as gastroenteritis, hepatoenteritis, respiratory symptoms, and skin irritation after consumption of contaminated drinking water or following recreational activities (Turner *et al.* 1990, Carmichael 1992a, Carmichael and Falconer 1993, Codd 1994, Ressom *et al.* 1994) and recently even deaths of haemodialysis patients due to cyanobacterial hepatotoxins in dialysis water have been reported (Carmichael 1996).

In addition to toxic secondary metabolites, cyanobacteria as well as other gram-negative bacteria produce lipopolysaccharides (LPS) as components of their outer cell wall. The lipopolysaccharides of cyanobacteria differ in their composition of lipid A from those of other gram-negative bacteria and also show weaker biological activity in *Limulus* assay (Keleti and Sykora 1982). Outbreaks of gastroenteritis and bathing fever due to cyanobacterial endotoxins in treated water have been suspected (Lippy and Erb 1976, Muittari *et al.* 1980).

1.1.1 Cyanobacterial hepatotoxins and their mode of action

1.1.1.1 Microcystins

Microcystins (MCYSTs) are monocyclic heptapeptides generally composed of D-alanine, D-*erythro*-β-methylaspartic acid (D-MeAsp), the novel amino acid (2S,3S,8S,9S)-3-amino-9-methoxy-10-phenyl-2,6,8,-trimethyldeca-4,6-dienoic acid (Adda), γ-linked D-glutamic acid, *N*-methyldehydroalanine (Mdha) and two variable L amino acids such as leucine(L), arginine(R), tyrosine(Y), alanine (A) or methionine(M), which are designated by two letter suffixes in different microcystins (Carmichael *et al.* 1988a). Since Botes and coworkers (1984) first determined the structure of cyanoginosin-LA from *M. aeruginosa*, almost 50 different microcystin compounds have hitherto been identified (Rinehart *et al.* 1994). Half of these different microcystins have been isolated from Finnish cyanobacterial strains or bloom samples (Sivonen *et al.* 1995). The main differences between these compounds are the variable L amino acids or the presence or absence of methyl groups on D-MeAsp and/or Mdha, or the replacement of the methoxy group of C-9 in Adda by acetoxy or hydroxy groups (Fig. 1). Adda has been shown to be a key structural component for biological activity (Dahlem 1989, Harada *et al.* 1990). The stereoisomers of Adda at the C-6 double bond, (6Z)microcystin-LR and (6Z)microcystin-RR, were non-toxic at doses up to 1.2 mg kg^{-1} in mouse bioassay with intraperitoneal injection, i.p. (Harada *et al.* 1990). (6E)Microcystin-LR and -RR had LD_{50}-values of 50 and 600 μg kg^{-1}, respectively. The range of LD_{50}-values of different microcystins is mainly from 50 to 250 μg kg^{-1} (i.p. mouse). The above-mentioned (6Z)isomers of microcystins and microcystins missing the free carboxyl group in glutamic acid are less toxic (Rinehart *et al.* 1994). Strains producing microcystins have hitherto been isolated from *M. aeruginosa, M. viridis, Anabaena flos-aquae, Anabaena spp., Oscillatoria agardhii, Nostoc sp.* and *Anabaenopsis milleri* (Sivonen 1996).

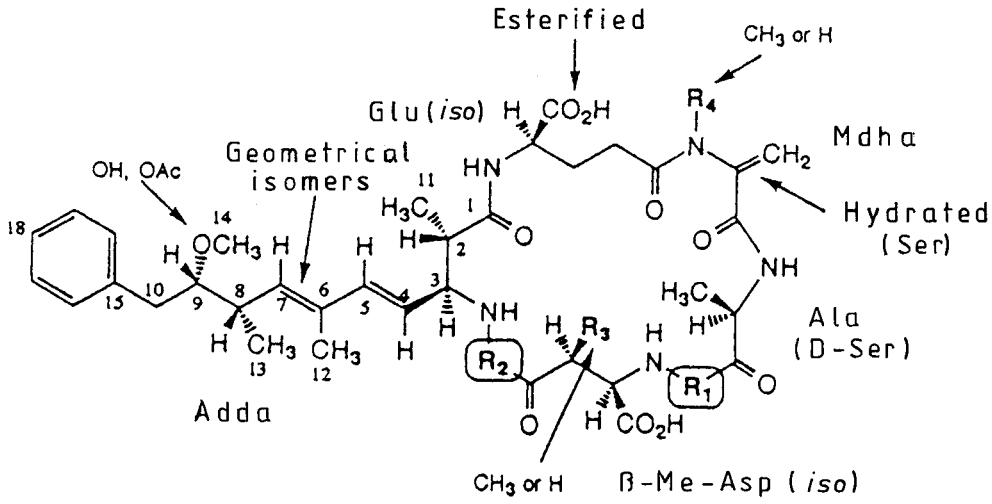

Fig. 1. The structures of different microcystins. R_1 and R_2 are variable L-amino acids (Structures based on data from Harada *et al.* 1994 and Sivonen *et al.* 1995).

The mechanism of toxicity of microcystins in cell and organ damage of mammalian organisms is now understood. In mammals, oral consumption of microcystins results in transport of the toxin through cell linings of the small intestine by bile acid transport system to the liver (Falconer *et al.* 1992). Microcystins also enter by the special uptake mechanism of bile acids to hepatocytes (Eriksson *et al.* 1990). In hepatocytes microcystins result in cell deformation and the development of blebs

(Runnegar *et al.* 1981, Eriksson *et al.* 1987). This deformation is due to breakdown of intermediate filaments of the cell cytoskeleton and reorganization of actin microfilaments (Eriksson *et al.* 1989, Falconer and Yeung 1992). Microcystins inhibit serine/threonine protein phosphatases 1 and 2A (Honkanen *et al.* 1990, MacKintosh *et al.* 1990, Yoshizawa *et al.* 1990), resulting in hyperphosphorylation of proteins associated with the cytoskeleton. Increased phosphorylation of intermediate filaments causes disassembling of filaments into dispersed spherical granules (Falconer and Yeung 1992). Phosphorylation of subunit proteins regulates the disassembly of intermediate filaments in normal cell mitosis, but microcystins disturb this regulation by phosphatase inhibition with consequent cytoskeletal disintegration (Falconer 1993a). The rapid loss of sinusoidal architecture leads to concentration of blood in the liver and hypovolemic shock (Runnegar and Falconer 1982, Hooser *et al.* 1989). The inhibition of protein phosphatases 1 and 2A by microcystins occurs in the nanomolar concentration range (Yoshizawa *et al.* 1990).

The effect of microcystins resembles the mechanisms of okadaic acid, a diarrhetic shellfish toxin produced by dinoflagellates, in specific phosphatase inhibition. Okadaic acid is a potent tumour promoter (Suganuma *et al.* 1988). The microcystin-induced hyperphosphorylation of the cytoskeleton results in a transition to an apparently mitotic state (Falconer and Yeung 1992) which relates to accelerated tissue growth (Falconer 1993a). Experiments with mice receiving toxic *Microcystis* in drinking water showed an increased weight of carcinogen-initiated skin tumours (Falconer and Buckley 1989). Potent liver tumour promotion after i.p. injection of microcystin-LR in rats was detected by Nishiwaki-Matsushima and co-workers (1992).

1.1.1.2 Nodularins

Nodularin (Fig. 2), produced by a brackish water species *Nodularia spumigena*, is a cyclic pentapeptide composed of Adda, β-linked D-*erythro*-β-methylaspartic acid, γ-linked D-glutamic acid, L-arginine and *N*-methyldehydro-α-aminobutyric acid (Rinehart *et al.* 1988). Seven different nodularins have been isolated from blooms or *N. spumigena* cultures and their LD_{50} -values range from 50 to over 2 000 μg kg^{-1} (i.p. mouse; Rinehart *et al.* 1994). In addition (L-Val^2)nodularin, named motuporin, has been isolated from a sponge found in Papua New Guinea (deSilva *et al.* 1992). Nodularins act in the same way as microcystins inhibiting protein phosphatase activity (Yoshizawa *et al.* 1990) and promoting tumours, but nodularin may also have tumour-initiating activity (Ohta *et al.* 1994).

1.1.1.3 Cylindrospermopsin

The most recently identified type of cyanobacterial hepatotoxin is cylindrospermopsin (Fig. 3), possessing a tricyclic guanidine moiety combined with hydroxymethyluracil (Ohtani *et al.* 1992). The toxin has been identified from a cyanobacterium *Cylindrospermopsis raciborskii* isolated from a water supply reservoir in Australia and from *Umezakia natans* at Lake Mikata in Japan (Ohtani

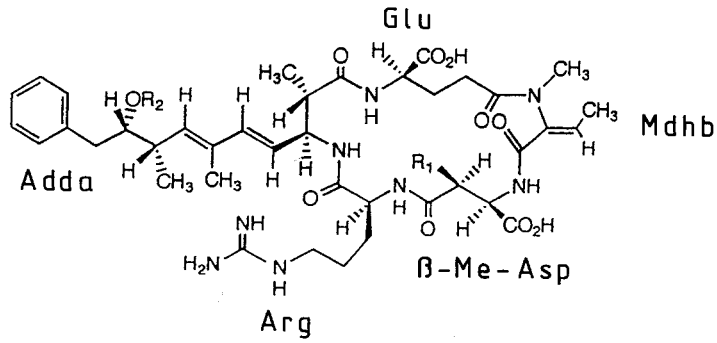

Fig. 2. The structures of different nodularins. R_1 and R_2 are methyl group or hydrogen (Redrawn from Rinehart *et al.* 1994)

et al. 1992, Harada *et al.* 1994). The main target organ of cylindrospermopsin is the liver but effects on thymus, kidneys and heart were also detected in experiments with mice (Terao *et al.* 1994). Cylindrospermopsin appears to be a potent inhibitor of protein synthesis (Terao *et al.* 1994). The LD_{50}-value to mice is 7 mg kg^{-1} (Harada *et al.* 1994).

1.1.2 Cyanobacterial neurotoxins and their mode of action

The first neurotoxin identified from the cyanobacterium *Anabaena flos-aquae* was called anatoxin-a (Huber 1972, Devlin *et al.* 1977). It is a bicyclic secondary amine, 2-acetyl-9-azabicyclo[4.2.1]-non-2-ene with a molecular weight of 165. It acts as a postsynaptic depolarizing neuromuscular blocking agent with an LD_{50}-value of 250 µg kg^{-1} body weight (i.p. mouse; Devlin *et al.* 1977). Death occurs within a few minutes and is due to respiratory arrest (Carmichael *et al.* 1975). Anatoxin-a is known to be produced by strains of *Anabaena flos-aquae, A. spiroides, A. circinalis, Aphanizomenon flos-aquae, Oscillatoria* spp., *Cylindrospermum* sp. (Sivonen *et al.* 1989a, Edwards *et al.* 1992) and *Microcystis aeruginosa* (Park *et al.* 1993a). Skulberg *et al.* (1992) isolated and determined the structure of homoanatoxin-a from *O. formosa.* The LD_{50}-value of homoanatoxin-a is 250 µg kg^{-1} body weight (i.p. mouse; Skulberg *et al.* 1992).

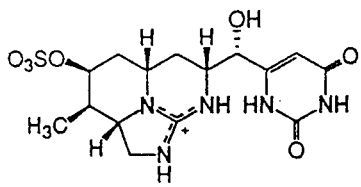

Fig. 3. The chemical structure of cylindrospermopsin (Redrawn from Ohtani *et al.* 1992).

Other identified cyanobacterial neurotoxins include anatoxin *a*(s), a potent inhibitor of cholinesterase (Mahmood and Carmichael 1986a, 1987), as well as paralytic shellfish toxins such as saxitoxin, neosaxitoxin and gonyautoxins also produced by dinoflagellates (Sasner *et al.* 1984, Mahmood and Carmichael 1986b, Humpage *et al.* 1994, Steffensen *et al.* 1994). Anatoxin *a*(s) is a phosphate ester of a cyclic N-hydroxyguanidine and it has been isolated from *Anabaena flos-aquae* (Matsunaga *et al.* 1989). Its LD_{50}-value for anatoxin *a*(s) is only 20 µg kg^{-1} body weight (i.p. mouse; Carmichael 1992b). Saxitoxin and neosaxitoxin inhibit nerve connections by blocking sodium channels (Adelman *et al.*1982). *Aphanizomenon flos-aquae* and *Anabaena circinalis* are the species of cyanobacteria known to produce saxitoxin, neosaxitoxin and gonyautoxins (Mahmood and Carmichael 1986b, Steffensen *et al.* 1994). The LD_{50}-values for saxitoxin and neosaxitoxin are about 10 µg kg^{-1} body weight (i.p. mouse; Mahmood and Carmichael 1986b).

1.2 Cyanobacterial toxins and human health

Human illnesses due to toxic cyanobacteria are far less widely reported than poisonings of animals. There are a few reports of outbreaks in which toxic cyanobacteria in raw water reservoirs have been implicated as the cause of illnesses or clinical symptoms through drinking water, but exhaustive epidemiological studies in areas with a high risk of cyanobacterial toxins in water supplies are missing (Falconer 1994, Hunter 1994). Bathing, canoeing or accidental swallowing of water with numerous toxic cyanobacteria has also been suspected to cause symptoms in humans (Dillenberg and Dehnel 1960, Turner *et al.* 1990). Cyanobacteria may cause symptoms through oral consumption, inhalation or skin contact.

1.2.1 Drinking water

Intestinal disorders in humans during the 1930-1931 drought in five states of Ohio and in the drainage basins of the Potomac rivers were associated with massive algal growth, musty odour and a heavy load of organic matter in raw water sources (Tisdale 1931). Repeated seasonal outbreaks of gastroenteritis among European children in Salisbury, Rhodesia, have also been attributed to decaying *Microcystis aeruginosa* blooms in lake McIlwaine during cold months, whereas the coloured population with another water supply did not have winter epidemics or problems with algae (Zilberg 1966; Weir, personal comm. ref. by Carmichael and Falconer 1993). In Sewickley, Pennsylvania an outbreak of gastroenteritis in late August 1975 affected some 5 000 people supplied by an open finished water reservoir with a mass occurrence of *Schizothrix calici-*

cola (Lippy and Erb 1976). Lipopolysaccharides of the filamentous cyanobacteria were suspected as one causative agent of the outbreak (Keleti *et al.* 1979), but there were also other possible causes due to deficiencies in reservoirs and raw water intake (Lippy and Erb 1976). A severe outbreak of hepatoenteritis in Palm Island, Australia affected 139 children and 10 adults shortly after a cyanobacterial bloom in a raw water supply of Salomon Dam was destroyed with copper sulphate (Bourke *et al.* 1983). Many of the affected children required hospital treatment for between 4 and 26 days before recovery from vomiting, liver enlargement, glycosuria, proteinuria, acidosis and bloody diarrhea (Byth 1980). In later studies the cyanobacterial strain cultured from the reservoir was identified as *Cylindrospermopsis raciborskii*, which caused death of mice due to hepatocyte necrosis and injuries in lungs, kidneys and intestine (Hawkins *et al.* 1985). The toxin was recently isolated and identified as an alkaloid hepatotoxin, cylindrospermopsin (Ohtani *et al.* 1992).

Falconer *et al.* (1983) reported a retrospective study of elevated liver enzyme activity, γ-glutamyl-transferase (GGT), in patients receiving their drinking water from Malpas Dam (Armidale, Australia) during mass occurrence of hepatotoxic *M. aeruginosa* compared to patients having other drinking water sources. The bloom was destroyed with copper sulphate. Three to eight weeks thereafter the enzyme activities of patients in the city were similar to the activities of control group subjects. This study has been criticized by lack of controlling the two populations (age differences) and lack of linking of the elevation of GGT with the dose of water consumption (Hunter 1994).

The high incidence of primary liver cancer in certain parts of China has been associated in epidemiological studies with the consumption of pond or ditch water instead of well water as potable water (Yu 1989). In Qidong, 1973–1982 the mortality from primary liver cancer was 61 per 100 000 among people drinking ditch or pond water and only 5.9 among people drinking water from deep wells (Yu 1989). Frequent occurrence of toxic *Microcystis* and *Oscillatoria* in these pond and ditch waters (Carmichael *et al.* 1988b, Ueno *et al.* 1996) has prompted surveys of quantitative data on microcystin in drinking water and epidemiological studies on the possible causative relationships between primary liver cancer and the amount of microcystin intake.

In February 1996 some 110 (84 %) of 131 patients at a haemodialysis centre in Caruaru, Brazil experienced visual disturbances, nausea and vomiting following a routine treatment (Carmichael 1996). During the next six months 44 of these patients had died of liver haemorrhage or liver failure due to microcystins. The water used for dialysis contained high levels of microcystins, due to a change in the source of raw water from the distributed water of the waterworks to natural lake water and an inadequently maintained in-house treatment facility (Carmichael 1996).

1.2.2 Recreational water

Dillenberg and Dehnel (1960) reported deaths of dogs, cattle and geese and also gastrointestinal symptoms in children after swimming in lakes during cyanobacterial blooms in summer 1959 in Saskatchewan. Cells resembling *Anabaena* were detected in a stool sample from one of the ten children suffering from diarrhea and vomiting on the following day after bathing in algae-covered lake water. A physician who accidentally swallowed cyanobacterial scum also suffered from nausea and stomach pains three hours after the accident, and a few hours later painful diarrhea followed by fever and pains in limb muscles and joints. *Anabaena circinalis* and *Microcystis* cells were detected in his faeces (Dillenberg and Dehnel 1960). Symptoms reported in humans during or less than 24 hours after contact with lake water containing *Anabaena* blooms have been eye irritation, earache, swollen lips, rash, sore throat, sneezing, nausea, vomiting and diarrhea (Billings 1981).

A toxic *M. aeruginosa* bloom in the Rudyard reservoir in Staffordhire, England, was probably the cause of pneumonia in two army recruits (Turner *et al.* 1990). The two soldiers together with eight other soldiers had been canoeing with rolls for several hours and swallowed small amounts of the bloom water. All of them suffered symptoms such as sore throat, headache, blistered mouth, abdominal pain, dry cough, diarrhea and vomiting. The two admitted to hospital with left basal pneumonia had pulmonary consolidation and low platelet count, features also detected in mice after microcystin intoxication (Slatkin *et al.* 1983). The soldiers recovered within a week. Inhalation was also suspected as a route of microcystin in-

toxication in this outbreak (Carmichael and Falconer 1993).

Experiments with intranasal administration of microcystin-LR in mice have shown toxicity equal to that after intraperitoneal injection, LD_{50} 250 μg kg^{-1}, whereas gastrointestinal administration was 12-fold less sensitive, LD_{50} 3 000 μg kg^{-1} (Fitzgeorge *et al.* 1994). In addition to liver lesions, microcystin-LR caused extensive necrosis of the epithelium of olfactory and respiratory zones via the intranasal route. A subacute dose of microcystin-LR increased the toxicity of anatoxin-a via the intranasal route. Particles of the same size (>10 μm in diameter) as in intranasal experiments with mice predominate in aerosols produced by waves, water-sports and swimming in natural waters and microcystins inhaled by the intranasal route may also affect mucosal surfaces in the bronchi and nasal areas in humans (Fitzgeorge *et al.* 1994).

Anabaena and phycocyanin may cause contact allergic dermatitis with red papules and vesicles and even more commonly skin irritation due to hydroxylamine produced during degradation of cyanobacteria (Cohen and Reif 1953). Heise (1949 and 1951, ref. Cohen and Reif 1953) reported hayfever-like symptoms in swimmers due to *Oscillatoria* and *Microcystis*. The literature published on allergic and irritative symptoms among swimmers in contact with mass occurrence of cyanobacteria does not confirm that microcystins or neurotoxins are the causative agents of these symptoms. On the contrary, some marine filamentous cyanobacteria such as *Lyngbya majuscula* occurring in tropical oceans cause severe burning and itching after bathing by producing phenolic bislactone toxins which are also potent tumour promoters (Moore 1981, Fujiki *et al.* 1984).

1.3 Concentrations of hepatotoxins in cyanobacteria and water sources

Quantitative data on cyanobacterial toxin concentrations in water bodies is rather sparse. Lindholm and Meriluoto (1991) detected some 20-30 μg l^{-1} desmethylmicrocystin-RR in the metalimnion of Lake Östra Kyrksundet during a mass occurrence of *Oscillatoria agardhii* (*Planktothrix agardhii*). The microcystin concentration in the epilimnion was simultaneously only a few μg l^{-1}. Microcystin was detected in the lake from the end of May to the beginning of September (Lindholm and Meriluoto 1991). Jones and Orr (1994) reported very high microcystin-LR concentrations, 990 and 1 830 μg l^{-1}, in Lake Centenary in southern Australia after algicide treatment of a *M. aeruginosa* bloom containing 8 mg g^{-1} toxin in dried cells. In Japan, total microcystin levels in different lakes varied during summer from less than 0.02 to 200 μg l^{-1} in the particulate fraction and from 0.02 to 2 μg l^{-1} in dissolved form (Tsuji *et al.* 1994b). The quantitative results presented above are based on measurements with high performance liquid chromatography.

In most studies, information on toxin amounts in dried cyanobacterial cell material collected from unknown volumes of bloom material is presented. Microcystin concentrations in cyanobacterial cell material varied from 25 to 2 100 μg g^{-1} in Japanese lakes during blooms dominated by *Microcystis aeruginosa* or *M. viridis* (Shirai *et al.* 1991, Watanabe *et al.* 1992b, Park *et al.* 1993a, b). Nodularin concentration in toxic bloom samples collected from the Baltic Sea varied from less than 0.1 to 18.1 mg g^{-1} (Sivonen *et al.* 1989b, Kononen *et al.* 1993). The hepatotoxin production in cyanobacteria appears to be affected by the light flux, temperature and the concentrations of phosphate, nitrogen, iron and zinc (van der Westhuizen *et al.* 1986, Sivonen 1990b, Utkilen and Gjølme 1992, 1995; Lukač and Aegerter 1993, Lehtimäki *et al.* 1994, Rapala *et al.* 1997). Different forms of microcystin toxins are produced under different light and temperature conditions (van der Westhuizen *et al.* 1986, Rapala *et al.* 1997). The amount of anatoxin-a in cultured cyanobacterial cells has been even above 1 % of the dry weight (Sivonen *et al.* 1989a, Rapala *et al.* 1993), and the amount of microcystins some 0.5 to 0.8 % of dry weight (Sivonen 1990b, Lukač and Aegerter 1993, Rapala *et al.* 1997).

1.4 Cyanobacteria, hepatotoxins and drinking water treatment

1.4.1 Control of cyanobacteria in reservoirs and raw water sources

Reduction of nutrient loading, especially from non-point sources is the most important and sustainable measure to control massive occurrence of cyanobacteria in watercourses. However, the beneficial effects of these measures may take a long

time and some additional measures for faster elimination of cyanobacteria are employed. Copper sulphate and other algicides have been and are still used in some countries to destroy massive occurrence of cyanobacteria in drinking water reservoirs (Carmichael 1992a). These chemicals as well as preoxidation with chlorine and potassium permanganate cause cyanobacterial cell lysis and sudden release of cyanobacterial toxins into the surrounding water (Kenefick *et al.* 1993, Lam *et al.* 1995b). Aluminium and ferric compounds are used to limit the growth of cyanobacteria by precipitating phosphates from raw water reservoirs. These compounds cause only minor release of microcystins from coagulated cells in water (Lam *et al.* 1995b, Peterson *et al.* 1995). Continuous mixing to break down stratification and aeration of water to decrease the amount of dissolved phosphorus have also been used (Hrudey and Lambert 1994). Biomanipulation is used for the elimination of cyanobacterial dominance in lakes either by harvesting planktivorous fish to increase the predation of zooplankton or by introducing algivorous fish such as carps which consume toxic cyanobacteria (Carmichael 1992a). Harvesting of planktivorous fish to eliminate cyanobacterial blooms has also been practised in Finland in different lakes (Peltonen and Horppila 1992, Sarvala *et al.* 1995).

1.4.2 Removal of cyanobacteria in treatment processes

No phytoplankton should be present in treated drinking water. Because total elimination is difficult, the aim of surface water treatment is to obtain over 99.9 % removal of algae (Janssens and Buekens 1993). Microstraining is often used as a pretreatment method to remove particles, such as large cyanobacterial colonies, from water. Direct filtration without any coagulant addition is used for oligotrophic waters but surface water treatment consists mainly of chemical coagulation, flocculation, flotation or sedimentation and rapid sand filtration processes. Chemicals used for coagulation of cells, such as aluminium sulphate and ferric chloride, efficiently remove large diatoms but the removal of small motile species, μ-algae (< 2 μm) and some filamentous species, such as *Oscillatoria*, is more difficult (Bernhardt and Clasen 1991). Phytoplankton removal in coagulation and flocculation varies from 50 to 99.9 % (Bernhardt and Clasen 1991). Flotation and preoxidation with ozone or potassium permanganate appear to improve the algal removal (Bernhardt and Clasen 1991, Petruševski *et al.* 1993).

1.4.3 Removal of microcystins in treatment processes

In laboratory scale experiments, dissolved hepatotoxins and anatoxin-a have passed the conventional surface water treatment processes based on coagulation, rapid sand filtration and disinfection with chlorine (Hoffman 1976, Keijola *et al.* 1988, Himberg *et al.* 1989). Powdered activated carbon in high concentrations and filtration through granular activated carbon have efficiently removed hepatotoxins and anatoxin-a from water (Hoffman 1976, Keijola *et al.* 1988, Falconer *et al.* 1989). Activated carbon produced from wood has a higher adsorption capacity for hepatotoxins than coal, peat or coconut-based activated carbon (Fawell *et al.* 1993, Donati *et al.* 1994). The adsorption capacity correlated positively with the mesopore volume of carbon particles (Donati *et al.* 1994). Contact time and competition for adsorption sites with other organic compounds present in water also affect the removal of toxins (Donati *et al.* 1994).

Studies on the effects of various oxidants on microcystins and anatoxin-a have shown that ozonation is an effective treatment method (Keijola *et al.* 1988, Himberg *et al.* 1989, Fawell *et al.* 1993). Ozonolysis of microcystin and nodularin resulted in cyclic peptide compounds without Adda, and neither these compounds nor the Adda alone showed any toxicity (Dahlem 1989). Sodium hypochlorite at a concentration of 1 mg Cl_2 l^{-1} and with a contact time of 10–15 minutes was ineffective against hepatotoxins (Himberg *et al.* 1989). Aqueous chlorine and calcium hypochlorite at 1 mg Cl_2 l^{-1} with low pH removed over 95 % of microcystins with a 30 min contact time (Nicholson *et al.* 1994). However, the use of high chlorine concentrations in drinking water treatment during mass occurrence of cyanobacteria induces high production of noxious organic chlorine compounds (Wachter and Andelman 1984, Wardlaw *et al.* 1991). Hydrogen peroxide was ineffective, whereas chlorine dioxide (6 mg l^{-1}) and potassium permanganate (2 mg l^{-1}) treatment of clarified wa-

ter resulted in 80 % removal of microcystin-LR (Fawell *et al.* 1993). Although UV-light destroys microcystins (Tsuji *et al.* 1995), its practical applicability to water treatment is limited due to the high irradiation intensities required (Drikas 1994). Nanofiltration through membranes with nominal molecular weight cut-off of 200 led to total rejection of microcystins (Fawell *et al.* 1993).

Biodegradation during slow sand filtration treatment resulted in moderate removal of both hepatotoxins and anatoxin-a but the efficiency varied in different trials (Keijola *et al.* 1988). Removal of cyanobacterial toxins has hitherto been studied mainly in laboratory and pilot plants and only a few studies are available from operated waterworks (Ohren 1988, Annadotter 1993).

1.5 Methods for monitoring of cyanobacterial toxicity and toxins

1.5.1 Bioassays

The method most widely used for monitoring cyanobacterial toxicity has been the mouse bioassay. Intraperitoneal injection of toxic cyanobacterial extract in mouse results in rapid death with symptoms typical of neurotoxin or hepatotoxin action (Beasley *et al.* 1989). However, the use of mouse bioassay has diminished due to ethical considerations as well as because of its low sensitivity (Falconer 1993b). The amount of cyanobacterial toxin needed to kill a 25 to 30 g Swiss Albino mouse is about 3 µg (Falconer 1993b). Mouse bioassay is suitable for cyanobacterial cell samples collected with plankton nets but not for the detection of toxins in raw or treated drinking water because large volumes of water need to be concentrated.

A bioassay using larvae of the brine shrimp *Artemia salina* was developed by Kiviranta *et al.* (1991b) for detection of cyanobacterial hepato- and neurotoxins. The *Artemia* bioassay gave results similar to those of mouse bioassay in 39 out of 44 bloom samples. However, in half of the laboratory-grown cyanobacterial strains (9/19), mortality of brine shrimp larvae due to subtances non-toxic to mice were detected with freeze-dried material. Another bioassay proposed for the screening of toxicity of cyanobacterial hepatotoxins is the bacterial bioluminescence assay (Lawton *et al.* 1990, Volterra *et al.* 1992). *Daphnia magna*, often used in toxicity bioassays, is sensitive to different axenic *Microcystis aeruginosa* strains but the toxic response does not correlate with mouse bioassay results obtained using the *Microcystis* strains (Nizan *et al.* 1986). *Daphnia pulicaria* also appears to be sensitive to some other compounds in *Microcystis* in addition to microcystins (Jungmann 1992).

1.5.2 Chemical and biochemical methods

High performance liquid chromatography (HPLC) methods for the detection of cyanobacterial hepatotoxins and anatoxin-a were developed towards the end of the 1980s (e.g. Krishnamurthy *et al.* 1986; Harada *et al.* 1988a, b, 1989; Meriluoto and Eriksson 1988). In most cases sample pretreatment for clean-up and concentration are needed. HPLC methods are important for identification, separation and quantitation purposes but they are not suitable for routine testing of cyanobacterial toxins. For concentrated bloom samples methods based on thin layer chromatography are also available (Poon *et al.* 1987, Ojanperä *et al.* 1991).

Biochemical methods for the detection of microcystins based on monoclonal and polyclonal antibodies have been developed (e.g. Kfir *et al.* 1986, Chu *et al.* 1989, Nagata *et al.* 1995) and some of them appear promising for routine analysis. The antisera produced against microcystin-LR showed good cross-reactivity with microcystin-LR, -RR, -YR and nodularin. The sensitivity of the assay was as low as 1 ng ml^{-1} (Chu *et al.* 1990). Methods based on protein phosphatase inhibition using radioactive phosphorus (Holmes 1991) are highly sensitive and they have recently been used for screening microcystins in lake and drinking water (Jones and Orr 1994, Lambert *et al.* 1994). This enzyme inhibition assay measures the actual toxic mechanism of microcystins and nodularin. Both the immunological methods and protein phosphatase inhibition assay give results as microcystin-LR equivalents.

1.6 Aims of this study

In Finland 44 % of the water distributed by public waterworks is surface water and an additional 9 % is artificial groundwater (Hatva 1996). In a survey from 1985 to 1987 on the frequency of toxic cyanobacterial blooms in Finnish freshwaters, 45 % of samples were toxic (Sivonen 1990a). The toxic samples included also bloom samples from 14

raw water sources of waterworks. The aim of this investigation was to evaluate the risks induced by mass occurrences of hepatotoxic cyanobacteria to the hygienic quality of drinking water in Finland and to assess methods for monitoring toxic cyanobacteria in raw water sources. This work focuses on the following aspects of the problem:

- removal of cyanobacteria and their toxins in different water treatment plants or processes (I, II, VI)
- assessment of simple methods for monitoring raw water toxicity (III)
- persistence and biological degradation of microcystins in water (IV, V)

2 Materials and methods

This investigation is based on studies conducted in operated waterworks and on laboratory and mesocosm experiments. In this section the main methods of the published studies are described briefly and more detailed information is given on the methods used to obtain the previously unpublished results.

2.1 Cyanobacterial cultures

The axenic and monocyanobacterial cultures used in the laboratory experiments are listed in Table 1. Most of these strains originated from the culture collection of Dr. K. Sivonen (Department of Applied Chemistry and Microbiology, University of Helsinki). *Synecococcus sp.*, isolated by Dr. M. Niemi, belong to the culture collection of Finnish Environment Institute. The strains were cultured in liquid Z8 medium (Kotai 1972) except N_2 fixing *Anabaena* strains, which were grown without added nitrogen at 20±2 °C for 2–4 weeks. Continuous illumination was provided by white fluorescent lights (Airam, Finland) at an irradiance incident on the surface of the growth vessels of 20 $\mu mol\ m^{-2}\ s^{-1}$. After incubation the cells in the cultures were lysed by freezing and thawing to release the toxins into the culture media. These treatment procedures were used in toxin degradation experiments (IV and this study) and in bioassays with neurotoxic *Anabaena* (III).

2.2 Phytoplankton analyses

Samples for phytoplankton analyses were taken from raw water (200 ml) and treated water (500 ml) in study II, a subsample of 200 ml from composite water samples of 4.5 l from lake water in mesocosm study V and 50 ml samples from raw and filtered water in study VI. The phytoplankton samples were preserved with Lugol and formaldehyde solutions and a drop of detergent was added to enhance sedimentation of cyanobacteria (Tikkanen 1986). The volume of samples decanted varied from 5 to 50 ml. Phytoplankton was identified and counted as cells, colonies or trichomes (filaments) with lengths of 100 µm (II, V) or 1 µm (VI) using an inverted microscope at magnifications of 200 and 800x. The number of algal cells was converted to biomass using the mean volumes of algal species obtained from the data bank of the Finnish Environment Institute.

2.3 Analyses of toxicity

The toxicity of concentrated cyanobacterial samples was determined with mouse bioassay as described by Sivonen *et al.* (1990) with fresh or freeze-dried cells (II). *Artemia salina* bioassay (Kiviranta *et al.* 1991b), luminescent bacteria test (Anon. 1991a) and *Pseudomonas putida* growth inhibition test (Anon 1991b) were evaluated for the detection of toxicity due to cyanobacterial hepato- and neurotoxins (III). In these bioassays solid phase fractionated extracts of hepatotoxic or non-toxic cyanobacterial blooms or cultures (Table 1, III) were used as test material. Solid phase fractionation was performed as described by Harada *et al.* (1988b) using Bond Elut C_{18} cartridges (Varian, USA). Both solid phase fractionated extracts and thawed, filtered solutions of cyanobacterial cultures containing anatoxin-a were used in the assessment of bioassays for detecting toxicity possibly caused by neurotoxins.

2.4 Cyanobacterial toxin analyses

Cyanobacterial hepato- and neurotoxins were in most cases determined by methods based on HPLC. Cyanobacterial cells were concentrated from known volumes of water and freeze-dried (II, V). The extraction and determination of hepatotoxins and anatoxin-a were performed as described by Meriluoto and Eriksson (1988) and Harada *et al.* (1989), respectively (II, V). Micro-

Table 1. Cyanobacterial strains used in laboratory experiments.

Strain	Source	Toxin	Reference
Anabaena sp. 202A1	Lake Vesijärvi	[Dha^7]MCYST-LR [Dha^7]MCYST-RR + two minor MCYSTs	Sivonen *et al.* 1992
Anabaena sp. 90	Lake Vesijärvi	MCYST-LR MCYST-RR [D-Asp^3]MCYST-LR	Sivonen *et al.* 1992
Microcystis aeruginosa 205	Lake Mallusjärvi	[Dha^7]MCYST-RR [D-Asp^3,Dha^7]MCYST-RR	Luukkainen *et al.*1994
Oscillatoria agardhii 97	Lake Maarianallas	[D-Asp^3]MCYST-RR	Luukkainen *et al.*1993
Anabaena flos-aquae 14	Lake Sääskjärvi	anatoxin-a	Sivonen *et al.* 1989a
Anabaena flos-aquae 37	Lake Sääskjärvi	anatoxin-a	Sivonen *et al.* 1989a
Anabaena circinalis 86	Lake Villikkalanjärvi	anatoxin-a	Sivonen *et al.* 1989a
Anabaena circinalis 123	Lake Säyhteenjärvi	anatoxin-a	Sivonen *et al.* 1989a
Anabaena mendotae 130	Lake Säyhteenjärvi	anatoxin-a	Rapala *et al.* 1993
Oscillatoria agardhii 156	Lake Koirajärvi	–	
Synecococcus sp.	Hurppu, Gulf of Finland	–	

cystins in cell material of raw water used for column experiments (VI) were extracted in water after freezing and thawing the cells on glass fibre filters (Whatman GF/C) from known volumes of raw water. Extraction and dissolution of toxins in distilled water was aided with sonication treatment. These samples as well as other filtered samples for the analysis of free hepatotoxins in water were concentrated and extracted with octadecylsilanized cartridges, Bond Elut C_{18} (Varian, USA) as described by Harada *et al.* (1988b) in studies III, IV, V and VI. An additional purification step with Sep Pak silica gel cartridges (Waters, USA) according to Tsuji *et al.* (1994b) was applied to fractionated extracts of toxins free in water in studies V and VI.

The hepatotoxin samples were analyzed with a Hewlett Packard 1090 High Performance Liquid Chromatograph with a diode array detector at 238 nm. In studies III and IV the column was a 3.9 x 150 mm μBondapak C_{18} (Waters, USA). The internal surface reverse phase column used in studies II and V was a Regis Pinkerton GFF-S5-80 (4.6 x 150 mm). In study VI the column was a 4.6 x 100 mm Hypersil ODS (Hewlett Packard, USA). The mobile phases and flow rates used are described in individual publications.

The toxins were identified by their retention

time, UV-spectra and addition of different purified microcystins. The toxin concentrations were determined by extrapolating peak areas to a calibration curve. The calibration curves for microcystins and anatoxin-a were determined by using purified [D-Asp^3]MCYST-RR, commercial MCYST-LR (Calbiochem, USA) and synthetic anatoxin-a (BioMetric Inc., USA).

Enzyme linked immunosorbent assay (ELISA) Envirogard^R Microcystins Plate Kit (Millipore, USA) based on polyclonal antibodies was used for microcystin detection of samples with toxin concentrations below the detection limit of the HPLC method (VI). The immunoassay method was later also used for preserved HPLC samples of particulate and dissolved toxins taken in 1993 and for samples of particulate toxins taken in 1994 during field experiments in lake Tuusulanjärvi. The immunoassays were performed according to manufacture's instructions and the results were read at 450 nm with a microplate reader (Dynatech MR1200, Great Britain). The detection limit for total microcystins with this immunoassay method was 0.1 μg l^{-1} for unconcentrated samples.

2.5 Operated waterworks

The information on waterborne outbreaks in Finnish community water systems (I) is based on reports from local health authorities to the National Board of Health, on personal communication with the national health authorities and on published case studies.

Removal of cyanobacteria, their toxins and other phytoplankton was studied in four Finnish surface water treatment plants during summer and autumn 1991. In two of the waterworks (Heinävesi and Taalintehdas) the basic treatment processes were rapid sand filtration and chlorination, in the Rymättylä waterworks contact filtration with aluminium sulphate and chlorination and in the Raisio–Naantali waterworks flocculation, sedimentation, sand filtration and chlorination. In three out of the four waterworks additional treatment with activated carbon powder or filtration through granular activated carbon was also practiced (Table 1, II). Water samples from the raw water were taken from pipes leading to the waterworks and for toxicity assays samples were also taken from the raw water sources near the intake site. Samples of treated water were taken from the water entering the distribution system. The frequency of sampling was once or twice a month.

2.6 Laboratory experiments

2.6.1 Persistence and biodegradation experiments

Laboratory experiments on the persistence and degradation of cyanobacterial toxins were performed in batch cultures with thawed and lysed extracts of cultured axenic or monocyanobacterial strains in Z8 medium (Kotai 1972). The persistence of toxins in water was determined either without additional inocula or after inoculation with lake water or sediment from different kinds of lakes (IV). The effect of adsorption on toxin occurrence was determined using sterile sediment inocula (IV).

In conjunction with field studies on toxin persistence (V), a sediment sample was taken in September 1994 from one of the mesocosm enclosures in lake Tuusulanjärvi. Subsamples of 0.16 g (dry weight) of this sediment were added to 250 ml conical flasks as inoculum to duplicate filtered (Whatman GF/C) solutions (100 ml) of thawed cultures of hepatotoxic *M. aeruginosa* 205 in Z8 medium. The total microcystin concentration in these solutions was about 4 mg l^{-1}. Two other solutions were prepared correspondingly after sterilization of the sediment samples twice in an autoclave (121 °C for 20 min) before inoculation. Two samples without sediment addition were used as controls. The sample bottles were transferred in the dark to 18±2 °C and incubated with rotation (135 rpm) for 16 days. During the incubation 5 ml samples were taken for toxin analysis by HPLC. After 8 days of incubation, sample solution from each bottle was inoculated to 10 % Z8 mineral medium with 1.2 % of agar together with fractionated extract of hepatotoxins from *M. aeruginosa* 205 as a sole carbon source. The microcystin concentration in the final Z8 agar medium was about 8 mg l^{-1}. The plates were incubated at 20±1 °C for 7 days. Strains of bacteria producing colonies on this medium were isolated and tested for their ability to degrade microcystins in liquid Z8 medium supplemented with fractionated extracts of microcystins from *M. aeruginosa* 205 (microcystin concentration 4 mg l^{-1}) for 7–10 days at 18±2 °C in the dark. Degradation ability was determined as a reduction of toxicity in the *Arte-*

mia assay (Kiviranta *et al.* 1991b).

The degradation rate of microcystins was determined with three different types of bacterial strains originating from the sediment of lake Tuusulanjärvi in 100 ml of Z8 medium together with solid phase fractionated extracts of microcystins from *M. aeruginosa* 205 (microcystin concentration about 10 mg l^{-1}) at 18±2 °C. Subsamples taken during incubation were analyzed for microcystin concentration by HPLC. A readdition of microcystin to cultures after 5 days of incubation was also performed to determine the rate of degradation in a situation in which degradation had already started. The numbers of bacteria during the experiments were determined by cultivation on R2A medium (Difco 1826) at 20±2 °C for 7 days.

2.6.2 Column experiments

The removal of cyanobacteria and their toxins from raw water in filtration was studied with soil and sediment columns (VI). The columns (height 0.3 m, diameter 0.1 m) were packed with soil from the surface layer of an esker used for artificial recharge of groundwater and with sediment sand from lake Vihnusjärvi at the site where natural bank filtration occurs. In the first experiment non-toxic *Oscillatoria agardhii* 156 together with pure, dissolved microcystin-LR and in the second experiment hepatotoxic *M. aeruginosa* 205 culture was suspended in water and filtered through the duplicate sediment columns and through a series of two soil columns for one week in the dark at 14±1 °C. The filtration rate was 0.038 m h^{-1} for the first three days in both experiments and thereafter 0.076 m h^{-1} in the *O. agardhii* experiment and 0.057 m h^{-1} in the *M. aeruginosa* experiment. The removal of cyanobacterial toxins and cells was monitored by analyzing samples taken from the raw and filtered water.

2.7 Field studies

Persistence of cyanobacterial hepatotoxins was investigated in lake Tuusulanjärvi during summer and autumn 1993 and 1994 with mesocosm enclosures (V). Polyethene enclosures (diameter 2.5 m, height 2.0 m) were fastened to the bottom at a depth of 1.2–1.4 m in a shallow bay. Half of the enclosures (one in 1993 and two in 1994) were black and they were covered to eliminate the effects of sunlight. Water samples from the enclosures and from the surrounding lake water were collected weekly for the analysis of toxins, phytoplankton composition, nutrients and other general water quality characteristics.

2.8 Statistical analyses

Correlation and partial correlation analysis were used to physical and chemical water quality results and log-transformed results of biological analyses. Differences in biomasses of different phytoplankton groups in samples taken near the surface and bottom in mesocosm experiments were compared with the paired t-test and differences in toxin concentrations in covered and uncovered mesocosm enclosures with analysis of variance (ANOVA). The decimal reduction time for microcystin-LR in water was determined with a linear regression model.

3 Results

3.1 Waterworks and treatment processes

3.1.1 Operated waterworks

Although most waterborne outbreaks reported in Finland during 1980–1992 were due to faecally contaminated water, mass occurrence of hepatotoxic cyanobacteria in raw water supplies of three waterworks diminished the quality of drinking water to such an extent that water was considered unsafe for human consumption (I). In all these cases cyanobacterial cells were detected in treated drinking water.

The removal of phytoplankton and cyanobacterial biomass varied in waterworks purifying surface waters with different treatment processes (Fig. 4, Table 2 and II). In waterworks without chemical coagulation, cyanobacterial biomass was removed less efficiently than total phytoplankton biomass. Filaments or cells of potentially toxic cyanobacteria from the genera of *Anabaena, Gomphosphaeria* and *Microcystis* were occasionally detected in treated water. The concentration of organic matter in treated water compared to the concentration in raw water remained almost un-

changed in rapid filtration plants (Table 2). In raw water chlorophyll *a* correlated positively with the biomass of total phytoplankton (r = 0.62, n = 38) and cyanobacteria (r = 0.65, n = 38) but in treated water no significant correlations between the same characteristics were detected.

One out of seven concentrated phytoplankton samples collected near to or from the raw water intakes of three waterworks was toxic in mouse bioassay (Table 4, II). The dominant species in the toxic sample was *Anabaena lemmermannii*. The compound responsible for neurotoxic symptoms remained unknown. The two litre samples taken simultaneously from treated water and raw water entering the waterworks were non-toxic in mouse bioassay. The phytoplankton composition in samples taken from the raw water source compared to the species composition of incoming raw water differed in the proportion of cyanobacteria (Table 4, II).

3.1.2 Filtration through soil and sediment columns

Filtration of water that contained non-toxic *O. agardhii* 156 and pure dissolved microcystin-LR through soil and sediment columns efficiently reduced both cyanobacterial filaments and toxin from percolated water (Table 3 and VI). In experiments with hepatotoxic *M. aeruginosa* 205, cells and toxins were retained less efficiently in soil and sediment columns than in the experiment with *O. agardhii* 156 (Table 3). In the experiments with *Microcystis* both cyanobacterial biomass and toxin concentration in raw water were about 200 times higher than in the experiment with *Oscillatoria.*

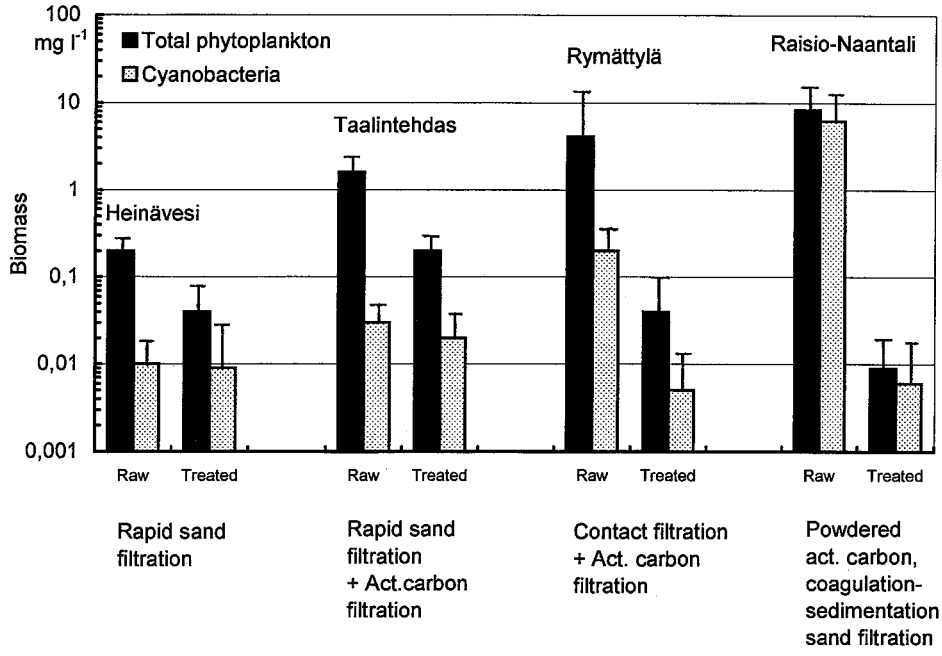

Fig. 4. The average total phytoplankton and cyanobacterial biomass in raw and treated water of different waterworks (bars indicate standard deviation, data from II).

Table 2. Average reduction of phytoplankton and cyanobacterial biomass, chlorophyll *a* and organic matter (measured as $KMnO_4$-value) in four operated waterworks (data from II).

Waterworks	Phytoplankton biomass	Cyanobacterial biomass	Chloro-phyll *a*	Organic matter
Rapid sand filtration				
Heinävesi	76 %	14 %	-2 %	5 %
Taalintehdas[1)]	88 %	42 %	85 %	19 %
Contact filtration				
Rymättylä[1)]	99 %	98 %	98 %	71 %
Flocculation-sedimentation-filtration				
Raisio-Naantali[2)]	99.9 %	99.9%	95 %	83 %

[1)]Activated carbon filtration as additional treatment
[2)]Addition of activated carbon powder into raw water during summer

Table 3. Reduction of cyanobacteria and microcystins from raw water after seven days' passage through a series of two soil columns[1)] and through two duplicate sediment columns (data from IV).

	REDUCTION %					
	Cyanobacterial cells			Microcystin conc.		
Column	Soil	Sed.I	Sed.II	Soil	Sed.I	Sed.II
O. agardhii experiment	99.999	99.92	99.91	97.93	99.15	99.27
M. aeruginosa experiment	90.54	No red.	98.21	91.08	50.39	60.61

[1)] The height of soil and sediment material in each column was 0.25 m.

3.2 Bioassays for monitoring cyanobacterial toxicity

The death of *Artemia salina* larvae responded to the concentration of hepatotoxins in the samples (Fig. 1, III). The LC 50 values for hepatotoxins varied from 3.7 to 17 mg l^{-1} (Table 2, III). The larvae were also sensitive to a neurotoxin, anatoxin-a, which affected their ability to move forwards. Anatoxin-a caused mortality in filtered solutions of cyanobacterial cultures but not in samples with fractionated anatoxin-a extracts (Table 3, III).

In the luminescent bacteria test the inhibition of luminescence did not systematically coincide with the concentrations of hepatotoxins or anatoxin-a in tested samples (Fig. 2, III). The growth of *Pseudomonas putida* was mostly enhanced by cyanobacterial toxin fractions (Fig.3, III).

3.3 Persistence and biodegradation of cyanobacterial toxins

3.3.1 Laboratory studies

Studies on the biodegradation of cyanobacterial toxins were performed with sediment and water inocula taken from lakes with different trophic status. Microcystins and anatoxin-a from thawed and lysed cultures of different laboratory strains

were degraded during the two-week study period (Figs. 1, 3 and Tables 1, 2, IV). Degradation of microcystins began sooner in samples with microbes from lakes with a recent history of cyanobacterial blooms than in samples with microbes from humic or oligotrophic lakes, in which degradation started after a lag period of 4 to 8 days. Degradation of anatoxin-a started equally rapidly

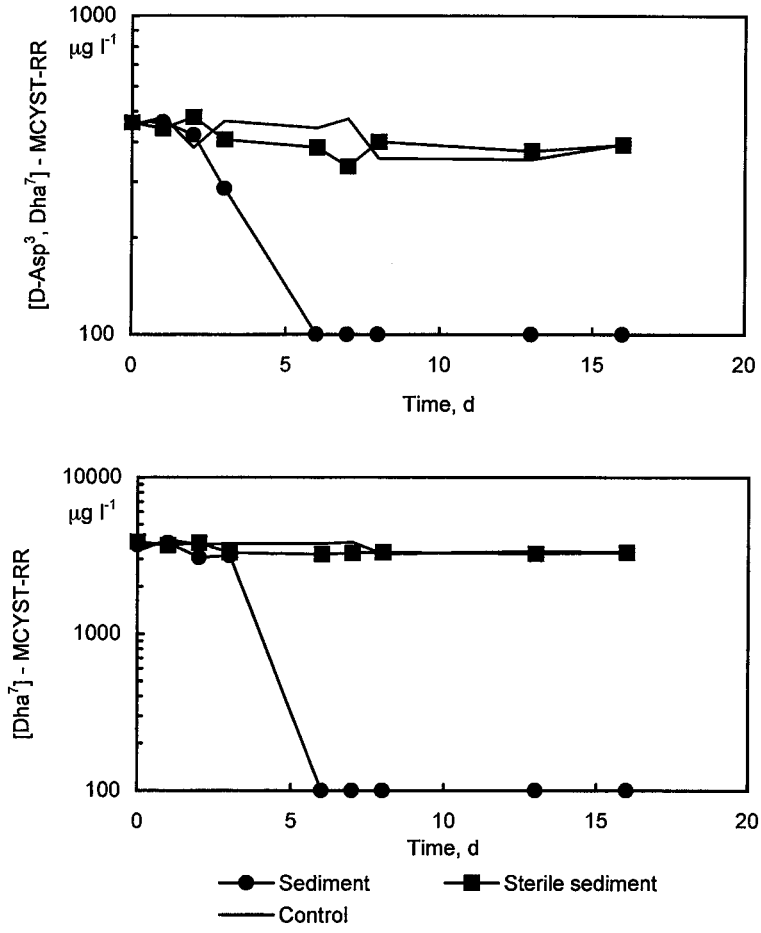

Fig. 5. Persistence of [D-Asp³, Dha⁷]-MCYST-RR and [Dha⁷]-MCYST-RR in water inoculated with natural and sterile sediment from lake Tuusulanjärvi (average of duplicate samples, coefficient of variation less than 25 % and 15 %, respectively).

with all the sediment inocula of different lakes (Fig. 3, IV).

The role of adsorption in toxin removal was studied by the addition of sterile sediment to crude toxin extracts. The adsorption of microcystins and anatoxin-a after 25 days was 10–35 % and 25–48 %, respectively (IV).

Bacteria present in the sediment sample taken from lake Tuusulanjärvi during field studies in 1994 degraded over 90 % of total microcystins, [D-Asp³,Dha⁷]microcystin-RR and [Dha⁷]microcystin-RR, in six days including a lag-period of at least two to three days before actual degradation (Fig. 5). A few strains of bacteria isolated from samples containing non-sterile sediment were able to degrade microcystins. The onset of degradation

varied with these strains from less than two to four days and 90 % removal was achieved after times ranging from 2.5 to over 5 days (Fig. 6). Reinoculation of microcystin to samples led to 90 % toxin removal after 10 to 40 hours depending on the strain tested (Fig. 6). The numbers of bacteria during the degradation experiments are shown in Table 4.

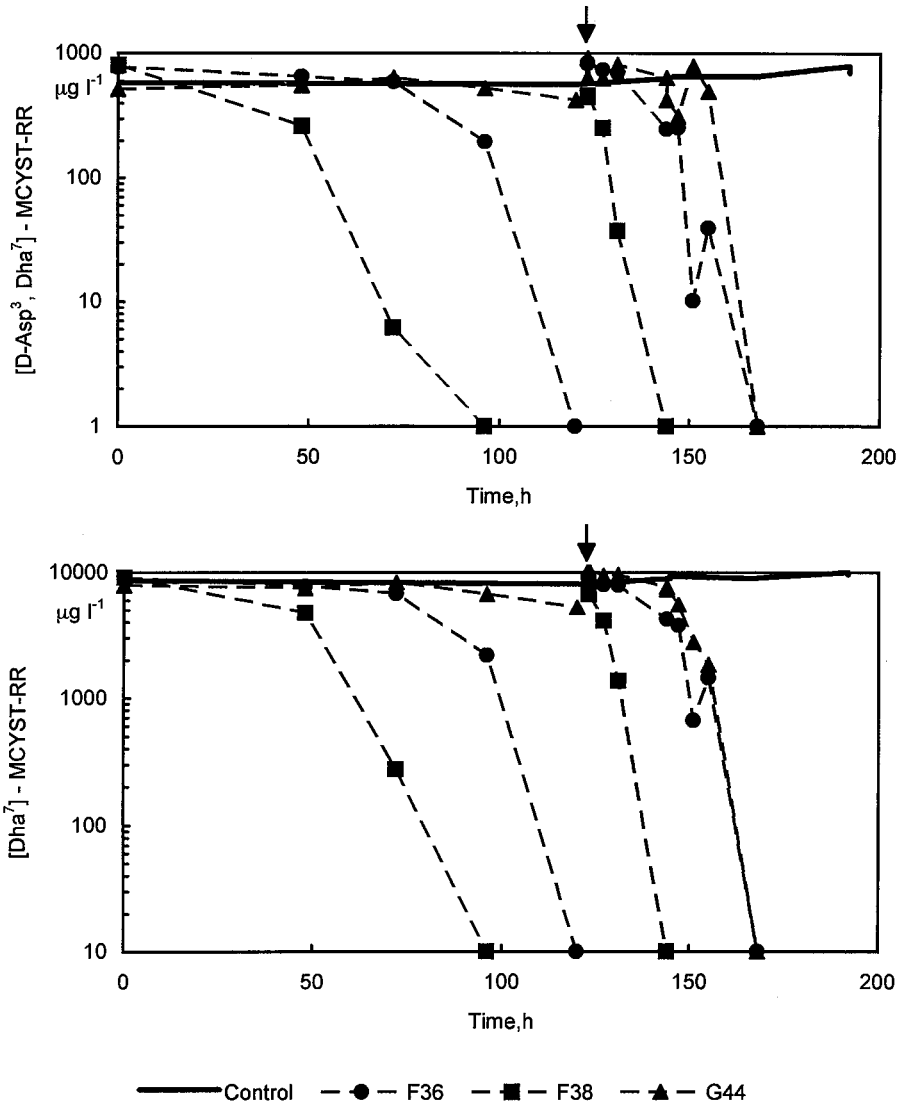

Fig. 6. Degradation of [D-Asp^3, Dha^7]-MCYST-RR and [Dha^7]-MCYST-RR in water by different bacterial isolates: F36, F38 and G44. The arrow indicates the time of readdition of toxin into the samples.

3.3.2 Field studies

During summer and autumn 1993 and 1994, persistence of microcystin was monitored in a small bay of lake Tuusulanjärvi. In August over 80 % of the phytoplankton biomass consisted of cyanobacteria (Fig. 2, V). *M. wesenbergii, M. viridis* and *M. aeruginosa* were the main species in 1993

but *M. wesenbergii* was the dominant species in August 1994 (Fig. 3, V). The cyanobacterial biomass in lake water in the beginning of the experiments was 7.5 mg l^{-1} in 1993 and 30.4 mg l^{-1} in 1994. However, the amount of microcystin-LR in cell material was higher in August 1993 than in August 1994 (Fig. 4, V). The maximum concentration of toxin in particulate material was 7.4 µg l^{-1} in 1993 and 3.2 µg l^{-1} in 1994 (Figs. 5 and 6, V). The water temperature decreased during the study periods from 16.0 °C to 9.0 °C in 1993 and from 20.9 °C to 8.2 °C in 1994. The oxygen and nutrient concentrations were rather similar during both years (Table 1, V). There was a significant positive correlation between microcystin-LR in particulate material and total cyanobacterial biomass, biomass of *M. wesenbergii* and total phosphorus during both years (Table 2, V).

Because the HPLC method was unable to detect dissolved microcystin from the 1993 samples, microcystin concentrations were later measured with the ELISA test based on polyclonal antibodies (Fig. 7). The concentration of dissolved microcystin-LR in lake water was from 0.2 µg l^{-1} to 0.01 µg l^{-1} in August and September 1993 and 1994. Microcystin concentration was also measured with the ELISA test from samples taken in 1993 and 1994 for toxins in particulate material (Fig. 7). These results showed significant positive correlation with the microcystin concentrations measured by HPLC ($r = 0.90$, $n = 18$).

Table 4. The numbers of microcystin degrading bacteria (cfu[1])/ml±95 % confidence interval) determined on R2A medium at 20±1 °C during experiments on microcystin degradation.

	Bacterial strains		
Time, h	F-36	F-38	G-44
0	$(3.2\pm0.76)\times10^4$	$(1.4\pm0.16)\times10^5$	$(2.2\pm0.20)\times10^5$
72	$(2.9\pm0.23)\times10^7$	$(1.7\pm0.18)\times10^8$	$(4.9\pm0.40)\times10^7$
120	Readdition of microcystin		
123	$(1.6\pm0.24)\times10^6$	$(4.0\pm0.28)\times10^7$	$(7.5\pm3.9)\times10^5$
171	$(2.3\pm0.68)\times10^7$	$(1.4\pm0.17)\times10^8$	$(2.7\pm0.22)\times10^7$

[1] cfu = colony forming unit

Microcystin concentration varied during the 1993 study period more than in 1994 due to a storm and an increase in phytoplankton biomass in early September. The decimal reduction times were therefore calculated separately for different periods in 1993 (Table 5). In 1994 the decimal reduction time for microcystin-LR in particulate material in lake Tuusulanjärvi was about 15 days and for dissolved microcystin-LR about one month (Table 5).

During the first two weeks in 1993 the concentration of microcystin-LR in particulate material was significantly higher in the covered compared to the uncovered mesocosm enclosure ($F = 6.79$, $P < 0.05$; Figs. 4 and 5, V). However, no significant differences in toxin persistence between covered and uncovered enclosures were detected in 1994 (Figs. 4 and 6, V).

4 Discussion

4.1 Efficiency of waterworks and treatment processes in the removal of cyanobacteria and microcystins

The poor removal of cyanobacteria and organic matter in the waterworks with rapid sand filtration and chlorination treatment without preceding chemical coagulation confirm the vulnerability of these waterworks to waterborne outbreaks of different origin (I, II). Although filtration through activated carbon is known to remove dissolved microcystin from water (Keijola *et al.* 1988, Falconer *et al.* 1989), intact cyanobacterial cells passed both the sand and activated carbon filters at the waterworks without chemical coagulation. The capacity of activated carbon to adsorb organ-

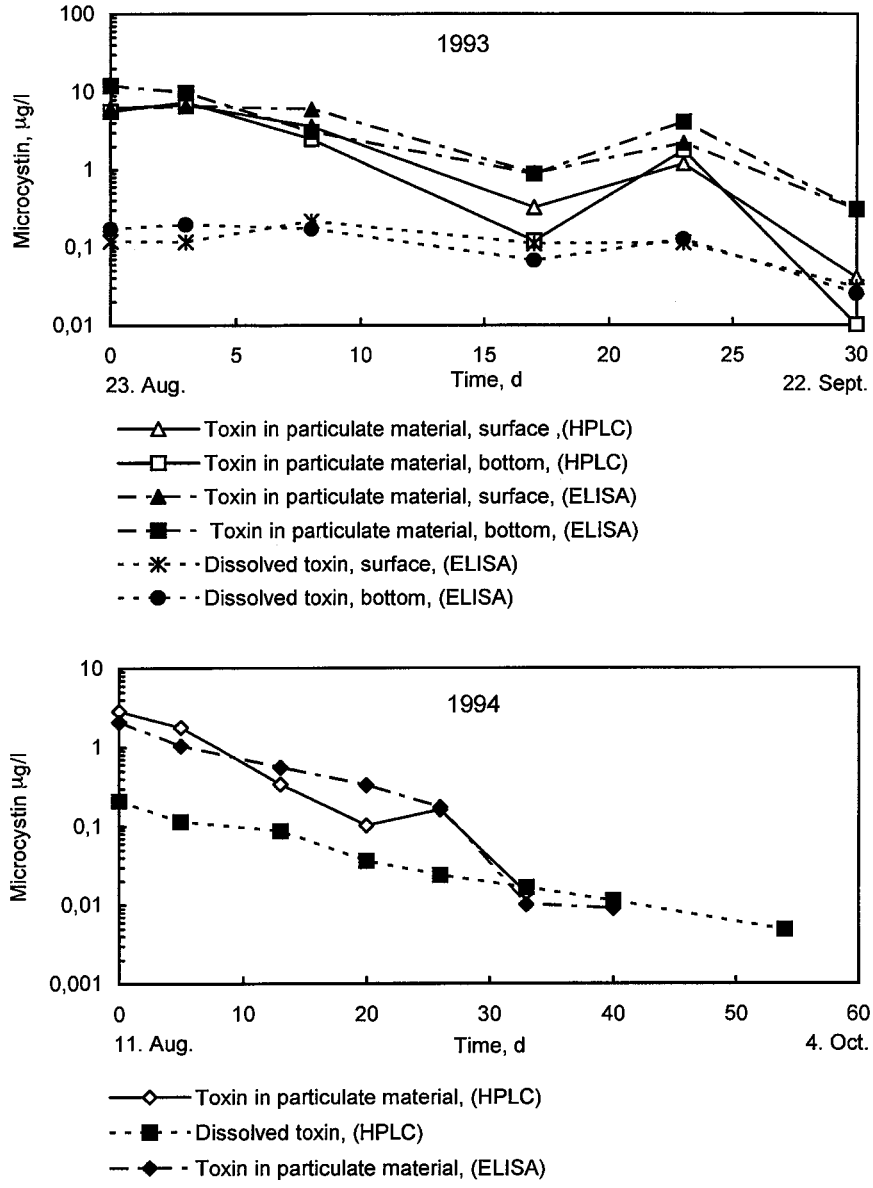

Fig. 7. Persistence of microcystin-LR in particulate matter and dissolved in water during August and September 1993 and 1994 in lake Tuusulanjärvi. Toxin concentrations were determined by HPLC and/or by immunological method (ELISA).

ic matter was probably exceeded due to the large amounts of particulate matter present in the water. This kind of water treatment procedure was probably the major contributing factor for microcystin occurrence in dialysis water and the outbreak of human poisoning in Brazil reported by Carmichael (1996).

Because most of the microcystins are inside the cyanobacterial cells during the exponential and stationary growth phase (Codd *et al.* 1989, Sivonen 1990b, Kiviranta *et al.* 1991a), efficient removal of intact cells eliminates most of the toxins. The average removal of phytoplankton and cyanobacterial biomass in waterworks with con-

Table 5. Decimal reduction time (T_{90}) for dissolved and particulate microcystin-LR calculated from the linear regression models based on results from lake Tuusulanjärvi in 1993 and 1994. In the equations C is ^{10}log of microcystin-LR concentration (μg l^{-1}) and T is the corresponding time in days. r^2 is the coefficient of explanation.

Linear regression model	T_{90} (d)	r^2
August 23 - September 22, 1993		
MCYST-LR in particulate material		
C = -0.0731T + 0.9630	14	0.729
Dissolved MCYST-LR[1)]		
C = -0.0204T – 0.7141	49	0.590
August 23 - September 9, 1993		
MCYST-LR in particulate material		
C = -0.0930T + 0.9996	11	0.886
August 31 - September 22, 1993		
Dissolved MCYST-LR[1)]		
C = -0.0333T – 0.6847	30	0.719
August 11 - October 4, 1994		
MCYST-LR in particulate material		
C = -0.0646T + 0.48511	15	0.929
Dissolved MCYST-LR		
C = -0.0300T – 0.7577	33	0.983

[1)] Based on results of immunoassay determination.

tact filtration was about 99 %, which is still below the 99.9 % removal set as a minimum goal for efficient treatment (Janssens and Buekens 1993). This reduction was only achieved at the waterworks with coagulation, flocculation and sedimentation treatment (II). However, during mass occurrence of cyanobacteria the number of cells may exceed 10^8–10^9 per litre. With 99.9 % removal efficiency treated water would still contain more than 10^5–10^6 cells per litre. Applying the data presented by Falconer *et al.* (1994) with a *M. aeruginosa* bloom and 0.2 pg microcystin per cell, this would correspond to a concentration of 0.02 to 0.2 μg microcystin l^{-1} in treated water. Destruction of cyanobacterial cells occurs during abstraction, microstraining, filtration and oxidation processes, thus still increasing the release of toxins into the water (Ohren 1988, Annadotter 1993, Dickens and Graham 1995, Lam *et al.* 1995b).

Infiltration experiments with soil columns suggested rather efficient removal of both cyanobacterial biomass and microcystin toxins even at high toxin concentrations (VI). Cyanobacteria and other phytoplankton were also effectively removed (99.9–100 %) at two waterworks using artificial recharge, but no information on hepatotoxin removal was obtained (Lahti *et al.* 1993). Toxin removal during bank filtration in operated waterworks has not been studied but results from column experiments with high toxin concentrations showed that microcystin removal was lower after filtration through sediment columns than through soil columns (VI). Although sediments from different kinds of lakes contained microbes which

were capable of toxin degradation in aerobic conditions (IV), efficient degradation was not detected in our sediment columns. The duration of column experiments was probably too short for efficient initiation of biodegradation in sediment columns, in addition to problems with overloading and breakthrough of cells. Further studies on microcystin removal in operated waterworks with bank filtration and artificial recharge are in progress.

The World Health Organization (1993) has not recommended any guide value for cyanobacterial toxins in drinking water because of the lack of sufficient data, but in Australia and Canada the proposed recommended upper limits for safe microcystin concentration in drinking water are 1 $\mu g\ l^{-1}$ and 0.5 $\mu g\ l^{-1}$, respectively (Falconer *et al.* 1994, Kuiper-Goodman *et al.* 1994). In recent surveys in the area of Haimen, known for its high incidence of liver cancer correlating with the use of drinking water from ponds and ditches (Yu 1989), the mean concentration of microcystins during the summer months in positive samples (17 %) was 0,101 $\mu g\ l^{-1}$ in pond and ditch waters, whereas in low wells the mean of positive samples (4 %) was 0.068 $\mu g\ l^{-1}$ (Ueno *et al.* 1996). In deep wells microcystin was not detectable. On the basis of these low microcystin concentrations and yet high incidence of liver cancer, probably due to the intake of the carcinogen aflatoxin B_1 from foodstuffs, the authors proposed an advisory level of 0.01 $\mu g\ l^{-1}$ for microcystin in safe drinking water. In the case of drinking water deterioration in the Taalintehdas community in 1989 the concentration of microcystin varied from 0.1 to 0.5 $\mu g\ l^{-1}$ (Lepistö *et al.* 1993). Recently Lambert *et al.* (1996) reported microcystin concentrations of 0.05 to 0.18 $\mu g\ l^{-1}$ assayed with the protein phosphatase inhibition method in treated waters from two Canadian waterworks with full scale coagulation-sedimentation treatment supplemented with either powdered or granular activated carbon treatment. The overall toxin removal ranged from 7 to 90 % and from 59 to 97 %, respectively, indicating the disturbance caused by other organic compounds present in water on the toxin adsorption capacity of activated carbon (Lambert *et al.* 1996).

During the past ten years additional treatment methods, such as activated carbon filtration, powdered activated carbon and ozonation have become more common in Finnish surface waterworks (Hiisvirta 1994, Vahala 1995). Theoretically these treatments combined with conventional treatment should safeguard potable water from high microcystin concentrations if maintained properly. However, overloading of filters due to long filter runs or exceptional mass occurrences of toxic cyanobacteria may endanger hygienic water quality. Chlorine (1 mg l^{-1} for 30 minutes) in other forms than sodium hypochlorite was assessed as an effective method for microcystin removal (Nicholson *et al.* 1994). Chlorination should not be used for microcystin removal because of the efficient formation of chlorinated organic substances from cyanobacterial compounds (Scully *et al.* 1988, Wardlaw *et al.* 1991), some of which are potential liver carcinogens (World Health Organization 1993). Even with low chlorine concentrations, high levels of adsorbable organic halogen compounds (AOX) have also been detected in treated water during summer in Finnish waterworks (Lahti *et al.* 1993). The best and most sustainable approach for minimising health risks due to cyanobacterial toxins is to continue the reduction of nutrient loading from point and non-point sources to raw water sources. This approach should reduce both the occurrence of cyanobacterial blooms and toxin production.

4.2 Persistence of microcystins in water

An important aspect of the health risk assessment of cyanobacterial toxins is the persistence of toxins in water. Persistence of microcystin-LR in lake Tuusulanjärvi appeared to be similar in 1993 and 1994: the decimal reduction times for particulate and dissolved toxin were about two weeks and one month, respectively (V). The persistence of both toxin types was rather long compared to the results from our laboratory experiments with sediment inocula or degrading bacteria, in which the decimal reduction time for microcystins was mainly less than one week. In laboratory studies with inocula from different surface waters or activated sludge, the 90 % reduction of microcystins varied from 3 to 40 days (Berg *et al.* 1987, Kiviranta *et al.* 1991a, Watanabe *et al.* 1992a, Kenefick *et al.* 1993, Jones *et al.* 1994, Lam *et al.* 1995a, b). In most cases the decimal reduction time was less than two weeks. These differences in persistence between laboratory and field results may be due to different temperatures, initial toxin concentrations, or the amount of other organic and inorganic nutrients and degrading bacteria present.

The temperature in laboratory experiments has usually been 18–25 °C, which is higher than the median temperatures of 12 and 15 °C during our field studies. However, the difference in toxin persistence of dissolved and particulate microcystin in lake Tuusulanjärvi appears to be controlled by other factors than temperature. The initial microcystin concentration both in our laboratory experiments and others has been several times higher than the concentrations of microcystin detected in lake Tuusulanjärvi. Jones and Orr (1994) detected a bi-phasic degradation kinetic of microcystin-LR in lake Centenary after algicide treatment with copper: rapid degradation from an initial toxin concentration of 1 830 to 110 μg l^{-1} with a decimal reduction time of 2.6 days was followed by a slower phase from 100 to 10 μg l^{-1} with a decimal reduction time of 15 days. The degradation was preceded by a lag phase of nine days. The persistence of microcystin in particulate matter in lake Tuusulanjärvi corresponded with that of the slower phase detected by Jones and Orr (1994). Because concentrations of dissolved microcystin were lower than 1 μg l^{-1}, their reduction probably occurs through co-metabolism with other compounds and by dilution. In earlier studies, dissolved microcystins were seldom detected in water and if detected the concentrations were only a few micrograms per litre or less (Watanabe *et al.* 1992b, Jones and Orr 1994, Tsuji *et al.* 1994b). Destruction of blooms with copper sulphate or rapid autolysis of cells may result in higher concentrations than those resulting from natural decomposition of blooms (Carmichael 1992a, Jones and Orr 1994).

The effects of sunlight on microcystin persistence in particulate material appear to be related more to the condition of cells than to actual toxin degradation (V). Although sunlight together with pigments from cyanobacteria and UV-light accelerated the decomposition of microcystins in laboratory experiments (Tsuji *et al.* 1994a, Tsuji *et al.* 1995), biodegradation of microcystins in the dark was also effective (IV).

A few strains of bacteria capable of microcystin degradation were isolated from the sediment samples. The degradation rates achieved by these strains corresponded to the rates detected in the actual sediment samples. The lag period preceding degradation varied with different strains, which might be due partly to the number of bacteria and production of enzymes, but also to the preference of other cyanobacterial compounds present in the toxin extracts. Jones *et al.*(1994) described a strain capable of degrading microcystin-LR and microcystin-RR but not nodularin. Their isolate resembles one of our strains, F-38, in its appearance, physiological characteristics (data not shown) and the rate of microcystin-RR degradation.

Persistence of cyanobacterial biomass and microcystin concentration in particulate material correlated positively in lake Tuusulanjärvi. When cyanobacterial biomass decreased below 1 mg l^{-1} (wet weight), the toxin concentration was below 1 μg l^{-1}. Whether or not this relationship also applies to other common hepatotoxic cyanobacteria, such as *Anabaena* and *Oscillatoria*, should be the topic of further studies.

4.3 Methods of monitoring cyanobacterial toxicity and toxins

Although cyanobacteria generally dominate in late summer and early autumn some cyanobacteria, such as *Oscillatoria*, may persist even under ice (Lindholm 1992). The occurrence of both toxin-producers and non-producers in the same species of cyanobacteria causes problems for monitoring. The other major difficulty for monitoring is the wide variation in toxin concentrations during short periods and between rather close locations in the same lake as detected in this study (II) and in other studies from Finland (Ekman-Ekebom *et al.* 1992, Lindholm *et al.* 1989) and from North America (Kotak *et al.* 1995).

Because the elimination of all phytoplankton from treated water is one of the goals, microscopical studies of treated water provide important information on treatment efficiency. Chlorophyll *a*, although sometimes exhibiting strong correlations with the occurrence of cyanobacteria (Lindholm *et al.* 1989), is not a suitable method for monitoring cyanobacteria or possible toxin occurrence in treated water due to its presence in all phytoplankton.

Monitoring of raw water quality with microscopical phytoplankton analysis and toxicity studies when cyanobacterial biomass is increasing has been the practice recommended by the Finnish health authorities to waterworks (Lääkintöhallitus 1990, Sosiaali- ja terveyshallitus 1991) and also by the authorities of other countries, e.g. Australia (Ressom *et al.* 1994). Screening of toxicity with

mouse bioassay needs special facilities and permission. *Artemia salina* bioassay appears simple and even specific if solid phase fractionation is performed. Also neurotoxic effects can be differentiated from hepatotoxic ones (III). However, concentrated samples are needed for the assay due to the rather high detection limit. The luminescent bacteria assay proposed for detection of cyanobacterial toxicity (Lawton *et al.*1990, Volterra *et al.* 1992), was not specific for cyanobacterial hepatotoxins or anatoxin-a (III). The lack of correlation between luminescence inhibition and the amount of microcystin-LR was also confirmed by Campbell *et al.* (1994).

In the case of toxic cyanobacteria, chemical toxin analysis from raw and treated water has been suggested. Chemical analysis of microcystins in raw waters and especially in treated water by HPLC requires expertise, large sample volumes, concentration and purification steps and different microcystin standards, only a few of which are commercially available. It is therefore not a suitable routine method for monitoring. New methods based on sensitive poly- or monoclonal antibodies appear to be promising tools for the determination of "total microcystin" concentration without tedious pretreatment of samples (Chu *et al.* 1990, An and Carmichael 1994, Nagata *et al.* 1995). The immunoassay correlated well with HPLC results in this study but this may partly be due to the sample preparation procedures. In natural lake water samples unspecific binding to antibodies may occur, especially when high concentrations of other organic matter are present (Chu *et al.* 1990).

Methods based on protein phosphatase inhibition appear also promising due to their high sensitivity, about 0.1 $\mu g\ l^{-1}$ for unconcentrated samples (Lambert *et al.* 1994, MacKintosh and MacKintosh 1994). Replacing radioactive phosphorus with a chromogenic substance (An and Carmichael 1994) would increase the usability of this assay although the sensitivity would be somewhat lower (MacKintosh and MacKintosh 1994). In the bioassays based on the inhibition of protein phosphatases, the effect of hepatotoxins may be masked due to endogenous phosphatase and proteolytic activities present in cyanobacterial samples and this should be taken into account by appropriate controls (Sim and Mudge 1994).

Monitoring of cyanobacterial biomass in raw water sources in combination with microscopical, bioassay and in some cases chemical methods appears reasonable. *Artemia* bioassay also provides information on the nature of toxicity in the case of cyanobacteria known to produce both neuro- and hepatotoxins. For monitoring the water treatment processes and for hepatotoxin occurrence in treated water, immunoassays and protein inhibition assays are promising but more studies comparing these methods with HPLC are needed to evaluate the specificities of the methods with natural water samples.

5 Conclusions

Removal of cyanobacterial biomass was low in surface waterworks without chemical coagulation. Mass occurrence of cyanobacteria in these waterworks poses a risk of microcystins in treated water because even intact cells may pass the treatment.

Although the average removal of cyanobacteria in waterworks with powdered activated carbon, chemical coagulation, sedimentation and sand filtration treatment was 99.9 %, problems in water quality during mass occurrence of cyanobacteria are possible. The general tendency towards more efficient removal of organic matter in Finnish waterworks will also be beneficial in the context of health aspects due to cyanobacterial toxins.

Efficient removal of microcystins from raw water by filtration through soil and sediment columns was detected except in cases of very high toxin concentration due to breakthrough of cells. Further studies on hepatotoxin removal, both in the field and the laboratory, are needed to assess the role of degradation and adsorption in the removal processes.

Bacteria capable of microcystin degradation occur in different lakes and sediments but the lag periods preceding degradation of microcystins appear to vary depending on the history of cyanobacterial dominance in phytoplankton biomass.

Persistence of microcystins in lake Tuusulanjärvi was longer than anticipated from the laboratory experiments. The decimal reduction time for microcystin-LR in particulate material was about two weeks and for dissolved toxin one month. The long persistence of microcystin-LR in water is an important factor to be considered when assessing the safety of water for drinking and recreational purposes.

The concentration of dissolved microcystin

during growth and decomposition of cyanobacterial biomass in lake Tuusulanjärvi was rather low, from a few nanograms to 200 ng l^{-1}.

Artemia salina bioassay is a promising method to replace the mouse bioassay for screening toxicity of cyanobacteria due to hepatotoxins and the neurotoxin anatoxin-a. The luminescent bacteria test was not suitable for screening toxicity of cyanobacterial hepatotoxins and anatoxin-a.

6 Summary

The aim of this study was to evaluate the risks due to mass occurrence of hepatotoxic cyanobacteria for the hygienic quality of drinking water in Finland, as well as some aspects of monitoring toxin occurrence in water sources. Surface water accounts for 44 % of water distributed by public waterworks and an additional 9 % is artificial groundwater. Toxic cyanobacteria have also been detected in raw water sources of waterworks.

According to laboratory and pilot studies, conventional surface water treatment processes pass most of the dissolved microcystins and the neurotoxin anatoxin-a to treated water. Although most of the waterborne outbreaks reported in Finland during 1980–1992 were due to faecally contaminated water, mass occurrence of hepatotoxic *Oscillatoria* in raw water supplies of three different waterworks resulted in temporary prohibition of the use of the water for human consumption. These waterworks had simple water treatment systems with only rapid sand filtration and disinfection with chlorine. Filaments of cyanobacteria, and in one case also microcystins, were detected in treated water.

In this study the efficiencies of operated waterworks in the removal of cyanobacteria and possible toxins were investigated in four different plants. Laboratory experiments were also performed in order to assess the removal of cyanobacterial cells and hepatotoxins during filtration through soil and sediment columns. The waterworks without chemical coagulation treatment were inefficient in the removal of phytoplankton and especially cyanobacteria from raw water, even when filtration through activated carbon was in use. The removal of dissolved organic matter was negligible. A contact filtration plant removed cyanobacteria and organic compounds more efficiently but problems in treated water are probable during mass occurrences of cyanobacteria. The average removal of cyanobacteria in waterworks with powdered activated carbon, chemical coagulation, sedimentation and sand filtration treatment, corresponding to the most common treatment processes used in Finnish surface waterworks, was 99.9 %. Even with these treatment systems problems in water quality during mass occurrence of cyanobacteria may occur in the absence of additional treatment methods.

Efficient removal of microcystins from raw water infiltrated through soil and sediment columns was detected except with very high biomass and toxin concentrations, probably due to breakthrough of cells. Further studies on hepatotoxin removal both in laboratory and in plants using artificial recharge of groundwater or bank filtration are needed to assess the efficiency of removal processes and the role of degradation and adsorption.

An important aspect of the health risk assessment of cyanobacterial toxins is the persistence of toxins in water and the role of biodegradation in toxin removal. Laboratory experiments with sediments collected from different lakes showed that bacteria capable of microcystin degradation occur in lake sediments and water, but that the lag periods preceding the onset of degradation of microcystins appear to vary. With sediments from lakes with a recent history of cyanobacterial blooms, degradation started faster than with sediments taken from a humic lake without numerous cyanobacteria. Some bacteria capable of degrading microcystins were isolated from the sediment of lake Tuusulanjärvi. These strains degraded microcystins as rapidly as the microbes present in added sediments, with 90 % removal in less than one week.

The studies on microcystin persistence in lake Tuusulanjärvi with covered and uncovered mesocosm enclosures and in lake water indicated much longer persistence of toxins than would have been expected from the laboratory experiments. The decimal reduction time for microcystin-LR in particulate material was about two weeks and for dissolved toxin one month. The long persistence of microcystin-LR in water is an important factor to be considered when assessing the safety of water for drinking and recreational purposes.

Dissolved microcystins have seldom been detected in water during cyanobacterial blooms. This may have resulted from deficiencies in sample clean-up methods, but it appears that even when detected the concentrations are rather low, e.g. in

lake Tuusulanjärvi from a few nanograms to 200 ng l^{-1}. New sensitive biochemical methods based on polyclonal antibodies hold promise for detection of low microcystin concentrations and may also prove to be useful tools for monitoring toxins in treated drinking water.

Simple, easy-to-use bioassay methods for the detection of toxicity of cyanobacterial blooms were compared in this study because of the need to replace mouse bioassays in toxicity tests. *Artemia salina* bioassay appeared promising for screening of toxicity of cyanobacteria due to hepatotoxins and the neurotoxin anatoxin-a. The luminescent bacteria test and the bioassay based on growth inhibition of *Pseudomonas putida* were not suitable for this purpose.

The results of this study and of other studies published in recent years indicate the need to monitor cyanobacterial toxins in water sources and drinking water and to promote methods for the reduction of blooms and toxins and their risks for public health.

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To my parents

Vantaa, March 1997 Kirsti Lahti

Yhteenveto

Tämän tutkimuksen tarkoituksena oli arvioida maksatoksisten syanobakteerien aiheuttamaa riskiä talousveden terveydelliselle laadulle Suomessa sekä toksiinien esiintymisen valvontaan liittyviä näkökohtia. Kunnallisten vesilaitosten jakamasta vedestä 44 % on käsiteltyä pintavettä ja lisäksi 9 % on tekopohjavettä. Myrkyllisten syanobakteerien massaesiintymiä on todettu myös osassa näiden vesilaitosten raakavesilähteitä.

Laboratorio- ja pilot-kokeiden perusteella tiedetään, että liuenneena olevat syanobakteerien tuottamat maksatoksiinit, mikrokystiinit, ja hermotoksiini, anatoksiini-a, kulkeutuvat perinteisten pintaveden käsittelyprosessien läpi. Vaikka valtaosa veden välityksellä levinneistä epidemioista Suomessa vuosina 1980–1992 aiheutuikin talousveden saastu-

misesta ulosteperäisillä mikrobeilla, jouduttiin kolmen vesilaitoksen jakaman veden käyttö väliaikaisesti kieltämään, koska raakavesilähteessä oli laaja rihmamaisen, maksatoksiineja tuottavan *Oscillatoria agardhii* syanobakteerilajin massaesiintymä. Näillä vesilaitoksilla oli vain yksinkertainen pikahiekkasuodatukseen perustuva vedenkäsittely ja klooridesinfiointi. Syanobakteeririhmoja, ja yhden laitoksen tapauksessa myös mikrokystiiniä, todettiin myös laitosten vesijohtovedessä.

Tämän tutkimuksen tarkoituksena oli arvioida syanobakteerien ja niiden mahdollisesti tuottamien toksiinien poistumista neljällä eri vesilaitoksella. Lisäksi tutkittiin laboratoriokokein syanobakteerisolujen ja mikrokystiinien poistumista imeytettäessä raakavettä harju- ja sedimenttipatsaiden läpi. Vesilaitoksilla, joissa vedenkäsittelyyn ei kuulunut kemiallista saostusta, kasviplanktonin ja etenkin syanobakteerien poisto vedestä oli heikkoa, vaikka käsittelyyn kuului lisänä suodatus rakeisen aktiivihiilen läpi. Liuenneiden orgaanisten yhdisteiden pitoisuus ei juurikaan laskenut vedenkäsittelyssä. Kontaktisuodatukseen perustuvalla laitoksella sekä syanobakteerien että orgaanisten yhdisteiden poisto oli selvästi tehokkaampaa kuin pikasuodatuslaitoksilla mutta tälläkin laitoksella ongelmat ovat todennäköisiä syanobakteerien massaesiintymien yhteydessä. Vesilaitoksella, jossa oli käytössä jauhemainen aktiivihiili, kemiallinen saostus ja selkeytys sekä hiekkasuodatus, syanobakteerien keskimääräinen poistotehokkuus oli 99.9 %. Kyseinen vedenkäsittely on yleisimmin käytössä Suomen pintavesilaitoksilla. Myös näillä käsittelymenetelmillä veden laatuongelmat ovat todennäköisiä massaesiintymien yhteydessä, ellei vedenkäsittelyä täydennetä lisämenetelmillä.

Raakaveden mikrokystiinipitoisuus laski huomattavasti suodattuessaan maa- ja sedimenttipatsaiden läpi, lukuunottamatta tilannetta, jossa käytettiin hyvin suurta syanobakteeribiomassaa ja siten myös korkeaa mikrokystiinipitoisuutta. Heikko poistuma aiheutui todennäköisesti oikovirtauksista, jolloin syanobakteerisoluja kulkeutui runsaasti patsaiden läpi. Lisätutkimukset sekä laboratoriossa että toimivilla tekopohjavesi- ja rantaimeytyslaitoksilla ovat tarpeen maksatoksiinien poistotehokkuuden arvioimiseksi imeytyksessä. Lisäksi tulisi selvittää adsorption ja biohajoavuuden merkitystä toksiinien poistossa.

Syanobakteeritoksiinien aiheuttaman terveysriskin arviointiin vaikuttaa merkittävästi toksiinien säilyvyys vedessä. Laboratoriokokeissa osoitettiin, että erityyppisistä järvistä kerätyissä sedimenttinäytteissä oli bakteereja, jotka pystyivät hajottamaan mikrokystiiniä, mutta hajotuksen alkaminen vaihteli eri näytteillä. Toksiinin hajotus alkoi nopeammin mikrokystiiniliuoksissa, joihin oli lisätty syanobakteerien massaesiintymän yhteydessä tai välittömästi sen jälkeen otettua sedimenttiä kuin sellaisissa liuoksissa, joihin lisättiin sedimenttiä humuspitoisesta tai karusta järvestä, jossa syanobakteerien massaesiintymiä ei esiintynyt. Tuusulanjärven sedimenttinäytteestä onnistuttiin eristämään muutamia bakteerikantoja, jotka hajottavat mikrokystiiniä. Näiden kantojen mikrokystiinin hajotus oli yhtä tehokasta kuin järvestä otetun sedimenttinäytteen eli pitoisuus laski 90 %:a alle viikossa.

Tuusulanjärvellä elo–lokakuun aikana 1993 ja 1994 suoritetuissa allaskokeissa mikrokystiini säilyi pimeissä ja avoimissa altaissa sekä vapaassa järvivedessä huomattavasti kauemmin kuin laboratoriokokeiden tulosten perusteella oli ennakoitavissa. Partikkeleihin pidättyneen mikrokystiinin pitoisuus laski kymmenenteen osaan noin kahdessa viikossa ja liuennut mikrokystiini vastaavasti noin kuukaudessa. Tällä mikrokystiinin pitkällä säilyvyydellä vedessä on huomattava merkitys arvioitaessa talousvesien ja uimavesien turvallisuutta, varsinkin näkyvän massaesiintymän hävittyä.

Syanobakteerien massaesiintymän yhteydessä on harvoin pystytty osoittamaan toksiineja liuenneena vedessä. Tämä on saattanut osittain johtua puutteista näytteiden puhdistuskäsittelyssä mutta näyttää myös ilmeiseltä, että liuenneiden toksiinien pitoisuudet vedessä ovat melko pieniä. Tuusulanjärvessä liuenneiden toksiinien pitoisuus oli muutamasta nanogrammasta 200 ng l^{-1}. Uudet herkät vasta-aineisiin perustuvat mikrokystiinien osoitusmenetelmät vaikuttavat lupaavilta myös liuenneiden toksiinien pitoisuuden määrittämiseksi sekä tarkkailumenetelmäksi vesilaitokselta lähtevän veden laadun valvontaan.

Syanobakteerien massaesiintymien myrkyllisyyden testaukseen tarvitaan hiiritestiä korvaava helppo ja yksinkertainen testi. Tätä varten arvioitiin *Artemia salina* -suolavesiäyriäisen käyttöön perustuvan biotestin sekä valobakteeritestin ja *Pseudomonas putida* -bakteerin kasvunestymistestin soveltuvuutta syanobakteerien tuottamien maksatoksiinien ja anatoksiini-a hermotoksiinin aiheuttaman myrkyllisyyden osoittamiseen. *Artemia salina* -biotesti osoittautui lupaavaksi hiiri-

testin korvaavaksi menetelmäksi, mutta valobakteeri- tai *P. putida* -bakteerin kasvunestymistesti eivät soveltuneet tähän tarkoitukseen.

Tässä tutkimuksessa saadut tulokset yhdessä viime vuosina julkaistujen tulosten kanssa osoittavat, että terveydellisen riskin vähentämiseksi on tarpeen seurata syanobakteeritoksiinien esiintymistä vesissä sekä edistää toimenpiteitä, joilla syanobakteerien massaesiintymiä vähennetään.

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